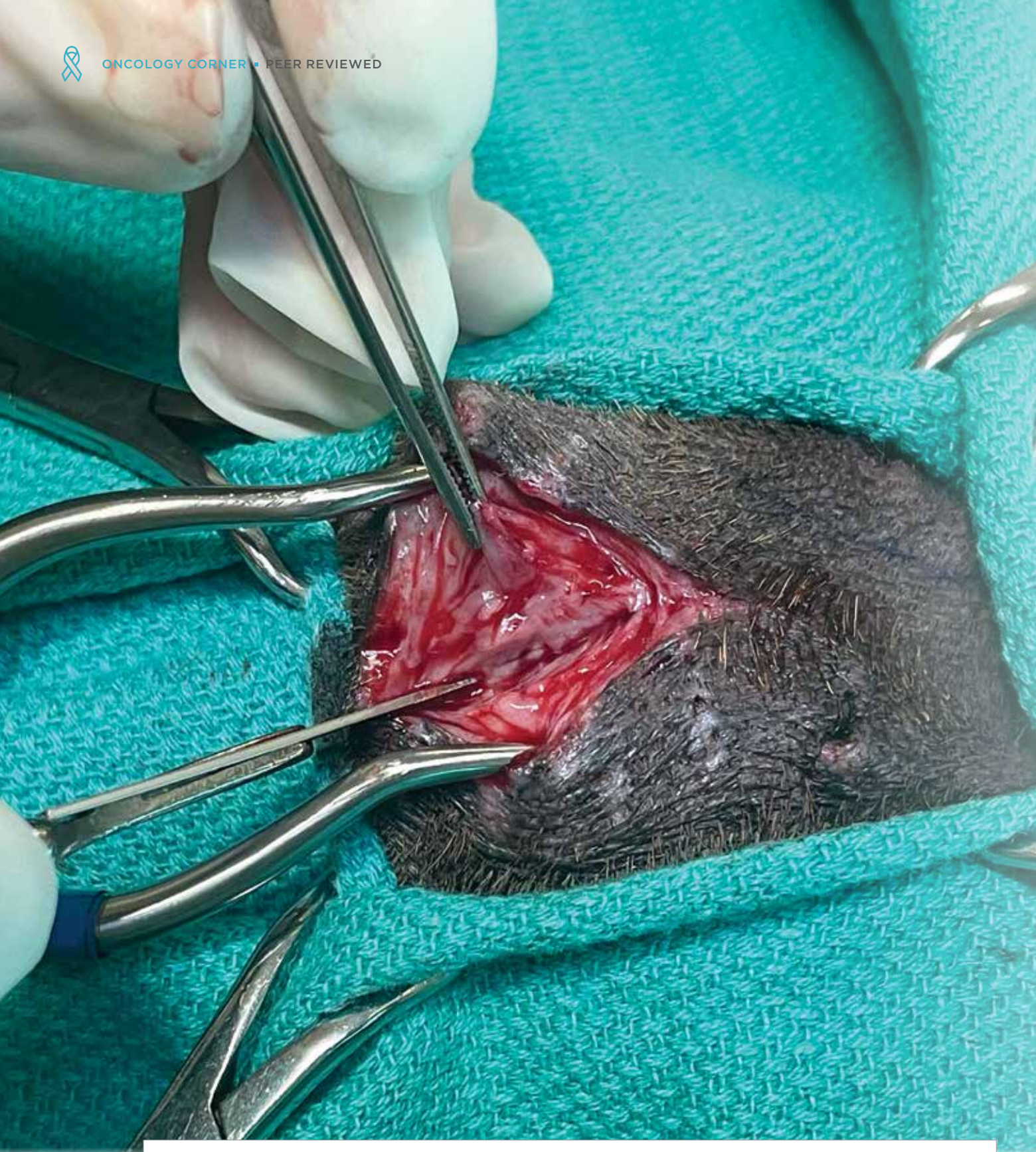




## Abstract

Obtaining an accurate preoperative diagnosis is imperative to properly stage and plan for surgery when treating dogs and cats affected by cancer. This article reviews biopsy of cutaneous and subcutaneous masses with emphasis on planning, indications, techniques, instrumentation, and potential complications.



## ONCOLOGY

# Biopsy of Skin and Subcutaneous Tumors: How to Maximize Success

**Brent Fink, MS, DVM**

**Giovanni Tremolada, DVM, MS, PhD, DACVS (SA), DECVS, ACVS Fellow, Surgical Oncology**  
Colorado State University College of Veterinary Medicine & Biomedical Sciences, Fort Collins, Colorado

Cutaneous masses account for approximately 5% of all medical concerns in small animal veterinary practice.<sup>1</sup> Obtaining a definitive diagnosis of a mass is an important first step to help guide future diagnostic or therapeutic strategies, manage owner expectations, and enable accurate communication of overall prognosis.

Tissue biopsy has long been a mainstay for diagnosing masses in veterinary medicine. Many sampling methods are available to veterinarians, ranging from fine-needle aspiration cytology (FNAC) to surgical excision (excisional biopsy). While not truly a biopsy technique, FNAC is usually considered the first-line diagnostic test for most cutaneous or subcutaneous masses.

Overall, the accuracy of diagnosing a lesion simply as malignant or not malignant from FNAC is >90%.<sup>2,3</sup> Escalation to tissue biopsy should be pursued if FNAC is acellular or nondiagnostic. Biopsy should also be considered if additional information about the tumor type would help direct treatment options or provide owners with useful prognostic information that may help future decision making.<sup>4</sup>

It is important to select a sampling method suitable to the clinical goals, anticipated results, and therapeutic plan, as inappropriate sampling techniques may complicate potential future therapeutic strategies.<sup>5</sup> Understanding the different biopsy techniques available, when they should be used, and their relative advantages and

## Take-Home Points

- Obtaining a definitive diagnosis before removal of cutaneous and subcutaneous tumors is typically indicated, as this will help determine extent of surgery for a planned mass removal.
- Different biopsy techniques have been described, and all have advantages and disadvantages.
- The surgeon should be familiar with these techniques and pick the most appropriate one for each individual lesion.
- Collecting multiple samples from a lesion, especially when samples are small, may increase the chance of reaching a reliable diagnosis.
- In certain cases, ultrasonography or computed tomography of the region affected by the mass can help with selection of a location for the biopsy.
- The biopsy tract should be planned so that it can be easily excised during the definitive surgery.



disadvantages are critical to ensuring the best outcome for patients with cutaneous and subcutaneous masses.

## CONSIDERATIONS FOR BIOPSY

The goal of a tumor biopsy is to collect a small tissue sample that is representative of the whole mass. While this may seem simple, biopsies can be nondiagnostic if an area of hemorrhage, necrotic tissue, or normal tissue superficial to the mass is sampled. Certain tumor types, such as soft tissue sarcomas, can become large with necrotic areas; therefore, obtaining a representative biopsy sample can be challenging.<sup>6</sup> In these cases, collecting multiple samples or a larger sample may increase the success rate of the procedure.

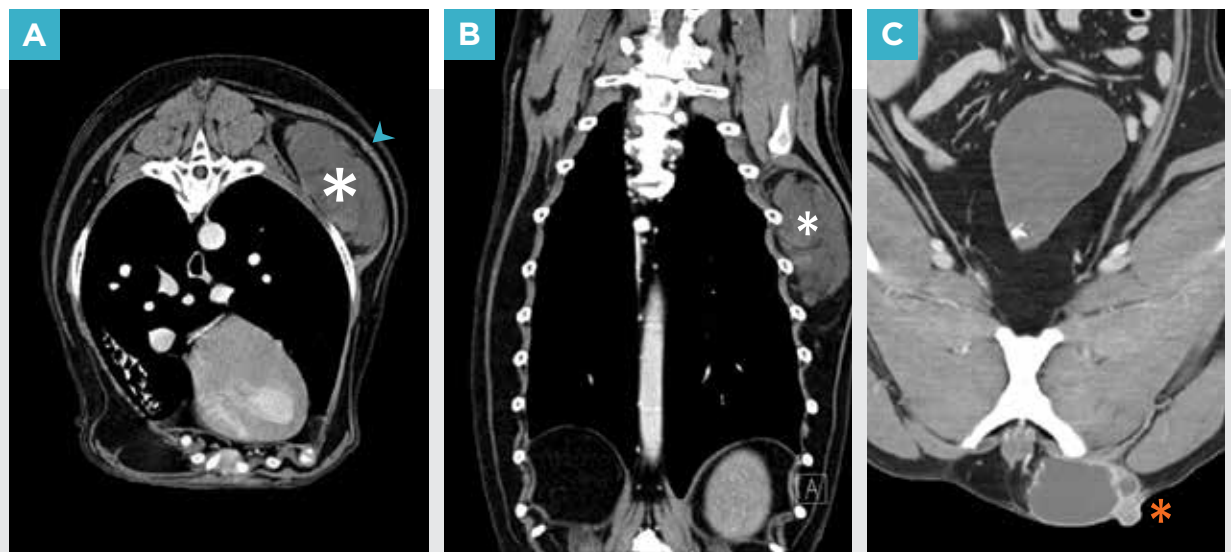
Performing ultrasonography or computed tomography (CT) of the affected area can help guide the clinician in selecting an appropriate biopsy location and reduce complications by avoiding areas of necrosis or highly vascularized regions. Imaging can also help in assessing the depth of the mass relative to the skin, allowing the surgeon to plan how much tissue dissection will be needed before reaching the tumor (**FIGURE 1**).

Specifically for soft tissue sarcomas, while biopsy is a reliable method of obtaining a correct histologic diagnosis, the concordance between tumor grade from the biopsy sample and final histology is only about

60%.<sup>7</sup> This degree of uncertainty should be communicated to owners, and information about the prognosis and potential need for adjunctive therapy should be delayed until a histologic grade from the entire mass is obtained.

For mast cell tumors, biopsies are often not required as FNAC is a successful, less invasive method of obtaining a diagnosis. An exception to this rule is if the tumor is going to be treated with tiludronate (Stelonta; Virbac, [vet-us.virbac.com](http://vet-us.virbac.com)). In these cases, it is important to determine the mast cell tumor grade via biopsy prior to treatment to decide if adjuvant therapy is indicated. For cutaneous mast cell tumors, a cytologic grading system has been described and could be used instead of a tissue biopsy but is not as accurate as a tissue biopsy.<sup>8</sup>

Tissue biopsy can be performed using a variety of techniques that are readily available in many first-opinion veterinary practices. Needle-core biopsy (Tru-Cut; Merit Medical Systems, [merit.com](http://merit.com)), punch biopsy, and wedge biopsy can all be used to obtain samples from cutaneous and subcutaneous tumors. Excisional biopsy (removal of an entire mass without prior identification of its type) should generally be avoided or reserved for cases where knowledge of the tumor type would not affect the treatment or otherwise limit future management options.<sup>6</sup>



**FIGURE 1.** (A) Transverse and (B) dorsal computed tomography (CT) images of a dog with a left thoracic soft tissue sarcoma (white asterisk) that is deep to the latissimus dorsi muscle (blue arrowhead). This muscle would need to be incised to obtain a representative sample of the underlying tumor. (C) Dorsal CT image of a dog with a necrotic soft tissue sarcoma. A blind biopsy in this case would have likely resulted in a nondiagnostic sample. Preoperative biopsy of the small nodule (orange asterisk) allowed a diagnosis of a grade 3 soft tissue sarcoma.



## CONSIDERATIONS FOR ANALGESIA

Tissue biopsy for cutaneous and subcutaneous masses can generally be performed with local anesthetic infiltration in patients under heavy sedation; however, general anesthesia can be considered if the patient is experiencing significant mass-associated pain, the mass is in a difficult location for sampling, or the patient has a comorbidity that makes general anesthesia a safer alternative.

Sedation or anesthetic protocols should provide adequate analgesia for the procedure, and local anesthetics (e.g., lidocaine, bupivacaine, liposome-encapsulated bupivacaine) should be used to improve patient comfort. Performing a local block and avoiding placing towel clamps when draping the surgical area may decrease the amount of discomfort created, allowing sampling to be performed under a lighter level of sedation. Postbiopsy analgesia and an Elizabethan collar should be provided, as pain or discomfort may lead patients to traumatize the biopsy site, which could further complicate definitive treatment. A short course (1 to 3 days) of a nonsteroidal anti-inflammatory drug, if not contraindicated, is usually enough to control postprocedural pain. If a mast cell tumor is suspected, administration of antihistamines before and for a couple of days after the biopsy is performed can be considered in hopes of reducing postprocedural inflammation due to mast cell degranulation.

## PLANNING FOR BIOPSY AND CLOSURE

Once the patient is adequately sedated, the biopsy site should be clipped, cleaned, and aseptically prepared. Sterile gloves and instruments should be used. A small

surgical pack consisting of a scalpel handle, blade, Metzenbaum/Mayo scissors, mosquito forceps, DeBakey or Brown-Adson forceps, towel clamps, a needle holder, sterile 4" × 4" gauze, and 4 quarter drapes are needed for the procedure.

When formulating a biopsy plan, it is imperative to understand that the biopsy tract itself will need to be removed at the time of the definitive surgery. Placing the biopsy site in an area that can be easily removed during the definitive surgery will increase the chance of obtaining complete surgical excision. Unlike biopsy for common dermatologic diseases, normal tissue at the margin of a mass should not be included as it will unnecessarily expand the proposed surgical area. Similarly, disrupting uninvolved fascial planes should be avoided as they can act as a barrier against deep spread of tumor cells. It is also important to consider the quality of the skin where the biopsy will be performed to ensure proper healing of the biopsy site. Regions with thin skin, skin that is tightly adhered to the tumor, and areas of ulceration should be avoided as these may not heal appropriately.

Closure of a biopsy tract can be done in 2 layers using small-gauge (3-0/4-0 USP), rapidly absorbable monofilament sutures on a taper needle for the subcutaneous tissue and 3-0/4-0 USP nonabsorbable monofilament sutures like nylon for the skin closure (**FIGURE 2**). For small biopsy sites, closure of the skin layer alone is an acceptable alternative. When a Tru-Cut needle biopsy is utilized, due to the small size of the skin incision performed to allow the insertion of the device, the skin defect can be allowed to heal by second intention or can be closed using skin glue. While larger sample sizes tend to increase the chance of getting a definitive histologic diagnosis, larger biopsy tracts also increase the risk for hematomas or seromas, increasing the possibility of seeding tumor cells in the surrounding tissues. Drains of any type are contraindicated when dealing with biopsies of tumors (and often after wide removal of tumors in general), as they may seed neoplastic cells into otherwise uninvolved tissues.<sup>4</sup>

## BIOPSY TECHNIQUES

### Needle-Core Biopsy

Needle-core biopsy instruments are relatively inexpensive and collect larger samples than FNAC (**FIGURE 3**). These instruments come in different sizes



**FIGURE 2.** Biopsy site closure.

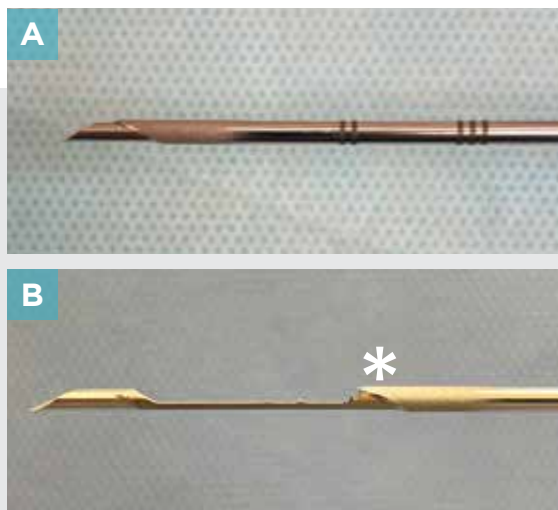


(most commonly 14G to 18G) and are typically labeled as single-use, although sterilization with plasma or ethylene oxide for reuse is common.

This biopsy method often produces a diagnostic sample, creates a small tract, and is especially indicated for masses in challenging locations (e.g., intra-abdominal, retrobulbar) or for masses with a large area of necrosis. In both instances, using ultrasound guidance improves both the safety of the patient and the chance to obtain a diagnostic sample. Penetration through uninvolved deep fascial planes should be avoided by approaching the mass parallel to the fascial plane (**FIGURE 4**) or by using ultrasonography to guide the instrument to an appropriate depth. While needle-core biopsy obtains smaller samples compared with other biopsy techniques, this technique is reported to provide an accurate histologic diagnosis in approximately 88% of cases.<sup>9</sup>

The basic steps of a needle-core biopsy are as follows:

1. Aseptically prepare the skin overlying the mass.
2. To avoid dulling the tip of the needle, make a small stab incision through the prepared skin and introduce the biopsy device through this incision.



**FIGURE 3.** Close-up image of the end of a needle-core biopsy device in the (A) “closed” and (B) “open” position. The device is introduced into the tumor in the closed position to avoid sampling unaffected tissues, decrease tissue trauma, and avoid bending the bottom part of the needle. Once the needle is inside the mass, pushing the trigger at the other end of the device advances the bottom portion of the needle. Applying additional pressure on the trigger causes the cutting sleeve (**asterisk**) to cut a core of tissue in the hollow part of the bottom portion of the needle. Care should be taken to remove the tissue sample from the device gently to reduce crush artifact.

3. Once the device is within the mass, fire the device to automatically collect a sample (**FIGURE 4**).
4. Remove the device.

Gentle handling of the sample is of paramount importance, as forceps or other instruments can cause a crush artifact that makes histologic interpretation of the tissue challenging for the pathologist. Using a small-gauge needle to remove the sample from the device notch (**FIGURE 5**) can help avoid this artifact. Collecting multiple samples by redirecting the instrument into different areas of the tumor through the same skin incision increases the chances of getting a diagnostic sample (**FIGURE 6**).

Because samples obtained with this technique are small, placing the sample in a specimen cassette instead of directly into formalin can help reduce the chance of additional fragmentation of the sample and loss of tissue architecture.

## Punch Biopsy

Punch biopsy is another inexpensive technique for



**FIGURE 4.** A needle-core biopsy device being introduced through a skin incision parallel to the patient’s skin. Introducing the device perpendicular to the skin could result in inadvertently disrupting the deep fascial layer, which could seed tumor cells into otherwise uninvolved tissues, or in damaging important deeper structures (e.g., the pulmonary parenchyma). The biopsy device can be reintroduced through the same skin incision in various directions to increase the chance of getting a diagnostic sample.



biopsy of cutaneous and subcutaneous masses. It utilizes a circular cutting blade on a handle and produces a shorter, wider tissue sample than a needle-core device (**FIGURE 7**). Punch biopsy instruments come in different sizes; the most frequently used have blades 2, 4, 6, or 8 mm in diameter. Since these instruments have a short cutting depth, sampling

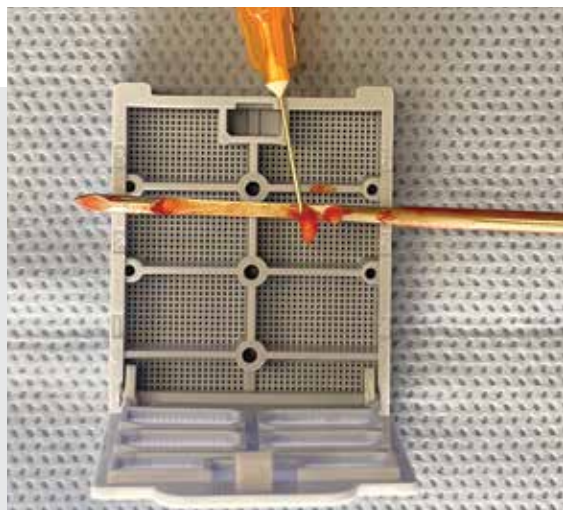
subcutaneous masses usually requires a skin incision with a scalpel blade to allow the instrument to reach the neoplastic tissue without sampling the overlying subcutaneous fat.

The basic steps of a punch biopsy are as follows:

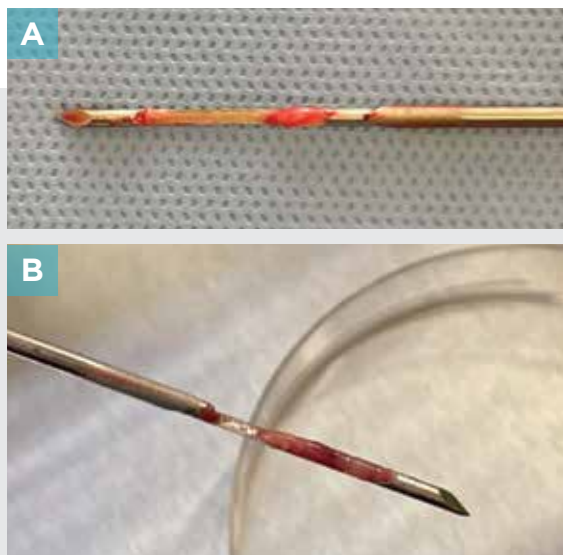
1. Once the surgical field is aseptically prepared, perform a local block and a skin incision.
2. Place the biopsy instrument over the area to be sampled and apply pressure to cut to the desired depth while twisting the device in a single direction. This allows the circular blade to create a core sample.
3. Free the sample from the mass by cutting its base with Metzenbaum scissors. For more friable/softer tumors, making a “scooping” movement with the instrument may be enough to separate the sample from the mass.

If the sample is retained inside the biopsy device, a sterile Kirschner wire can be fed through the hollow handle to retrieve it, preventing crush artifact. Forceps can also be used to retrieve the sample from the punch; however, this increases the chance of creating artifacts that may prevent a pathologist from making a diagnosis. As with needle-core biopsies, collecting multiple samples through the same skin incision helps increase the chances of getting a diagnostic sample. The samples can be placed in a cassette or directly in a formalin jar.

This method produces a larger defect than a needle-core biopsy; thus, appropriate hemostasis and wound



**FIGURE 5.** A 25G needle can be used to gently remove a needle-core sample to prevent crush artifact that can be caused by forceps. The quality of this sample is low, and an additional core should be obtained. Microcassettes with numbered wells can help keep samples organized when multiple masses are biopsied at the same time.



**FIGURE 6. (A)** An example of a low-quality needle-core sample. The left side of the core (pale/tan) is necrotic tissue; the pink/red portion is the proper mass. **(B)** Appropriate sample obtained with a needle-core device. Due to the small sample size, several samples should be obtained.



**FIGURE 7.** Appropriate-size samples obtained with a punch biopsy device. Multiple samples were collected, placed in a specimen cassette, and then immersed in 10% formalin. The mass was diagnosed as a rhabdomyosarcoma.



closure should be anticipated. Using electrosurgery for the initial skin incision or to control bleeding at the biopsy site can improve visualization and decrease the procedural time. Alternatively, gentle compression of the area with a sterile sponge can be used to obtain hemostasis. After all the samples are collected, the biopsy site is closed as previously described.

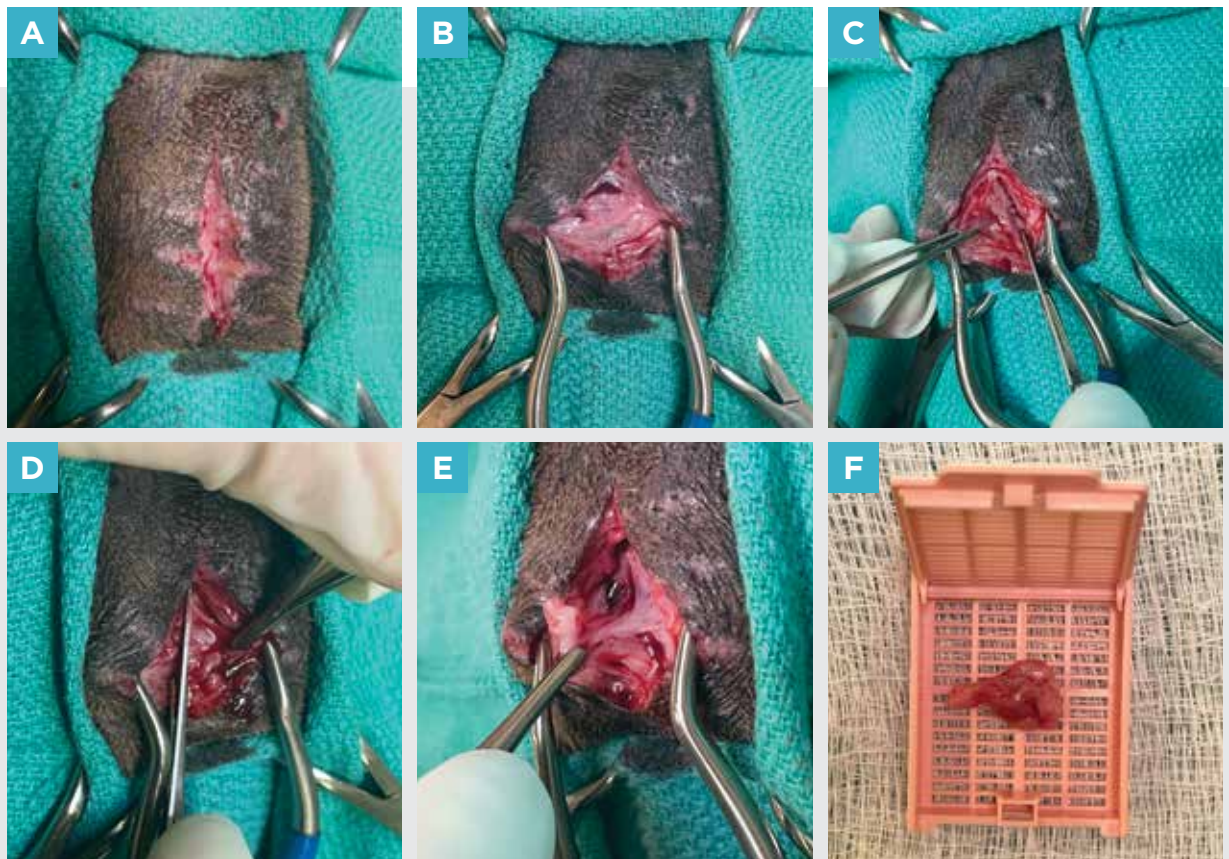
## Wedge Biopsy

Incisional biopsy is a direct and economical approach to tumor biopsy as it does not require any special instruments, making it essentially universally available. As with other techniques, an initial skin incision is required; however, the incision is larger, allowing for increased visualization and isolation of tumor tissue. Electrocautery can cause too much disruption of the tissue architecture (cautery artifact), particularly if the sample size is small, and its use in collecting samples

should be limited.<sup>4</sup> Electrocautery can be safely used to obtain hemostasis of the biopsy bed.

The basic steps of a wedge biopsy are as follows (**FIGURE 8**):

1. Aseptically prepare the skin overlying the mass.
2. Using a scalpel blade (#15 or #10 depending on the size of the mass and surgeon's preference), incise the prepared skin. Minimal dissection should be performed to expose the mass to reduce the risk of hematoma/seroma formation and subsequent potential seeding of neoplastic cells. If the tumor is subcutaneous, a Gelpi self-retaining retractor can be used to improve visualization.
3. Make 2 parallel incisions into the tumor that meet at the deep aspect of the sample, forming a wedge. In general, a deep, narrow wedge is preferred to a shallow, broad one as this tends to avoid accidental sampling of the tumor pseudocapsule.<sup>10</sup>



**FIGURE 8.** Steps of a wedge biopsy. **(A)** A skin incision is performed with a scalpel blade to access the subcutaneous mass. **(B)** A Gelpi retractor is used to retract the skin edges to better expose the mass. **(C)** An incision is made over the neoplastic tissue while lifting the tissue with Brown-Adson forceps. **(D)** A second incision is made a few millimeters away from the first, angling the blade to create a wedge. **(E)** The last attachments of the sample to the mass are visualized by pulling the biopsy upward and detached using a scalpel blade or a pair of Metzenbaum scissors. **(F)** The sample is placed in a cassette and put in a container with 10% buffered formalin.



As with other biopsy techniques, sample handling with forceps should be reduced to prevent crush artifact.

If there is any doubt about how the definitive surgical resection will be performed, the incisional biopsy should be done by the practitioner who will ultimately perform the resection. As with all biopsy techniques, care should be taken during the biopsy procedure to minimize seeding cancer cells into unaffected tissues or tissue planes. The downside of wedge biopsies is that, due to the larger size of the incision required to obtain a sample, they carry a higher potential for complications such as hemorrhage and infection than other techniques.

## BIOPSY SUBMISSION

In general, once a biopsy sample is obtained, it should be minimally handled to prevent artifact that could prevent diagnosis. Samples should be placed in 10% neutral buffered formalin within 30 minutes of biopsy.<sup>11</sup> A ratio of roughly 10 parts formalin to 1 part tissue sample by volume is ideal to ensure appropriate preservation. Small samples, such as those from needle-core devices and small biopsy punches (< 5 mm), should be placed into plastic cassettes to facilitate handling and prevent sample loss. Submission forms should include the animal's signalment, lesion description, rate of lesion progression, relevant complete blood count and serum biochemistry abnormalities, changes on imaging (e.g., radiographs, ultrasounds, CT images), and any previous histopathologic or cytologic diagnostic results.<sup>11</sup> Photos of the lesion before biopsy could be added to the submission form to provide clinical context and increase the odds of an accurate diagnosis.

Histopathologic results of a tumor biopsy should always be considered within the clinical context. If the pathological diagnosis does not fit the clinical history of the patient, reaching out to the pathologist to give additional information about the case or asking for a second pathologist opinion should always be considered.

## SUMMARY

Obtaining an accurate diagnosis is imperative to stage and plan for surgery when treating dogs and cats affected by cancer. Tissue biopsy can be performed using a variety of techniques, and it is important to select a sampling method suitable to the clinical goals, anticipated results, and therapeutic plan. **TVP**

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### Brent Fink

Dr. Fink earned his DVM degree from Colorado State University, where he is now a rotating intern at Colorado State University's James L. Voss Veterinary Teaching Hospital. Dr. Fink is interested in small animal surgery, with an emphasis on surgical oncology and orthopedics.



### Giovanni Tremolada

Dr. Tremolada is a clinical assistant professor of surgical oncology at Colorado State University. He received his DVM degree from the University of Milan, Italy, and completed a PhD program at the same institution. He started a combined residency and master of science degree in small animal surgery at Colorado State University and subsequently completed a surgical oncology fellowship at the Flint Animal Cancer Center. He became a diplomate of the American College of Veterinary Surgeons (ACVS) in 2019 and an ACVS Fellow in surgical oncology in 2020. In 2022, he also became a diplomate of the European College of Veterinary Surgeons.